PATHOLOGICAL CHANGES IN MURINE HEARTS INDUCED BY INTERMITTENT ADMINISTRATION OF CIGUATOXIN

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Ciguatoxin (CTX) at doses of 0,1 or 0,05μg/kg were given orally by intubation into male ICR mice once a week for 25 weeks (0.1μg/kg group) or 40 weeks (0.05μg/kg group). Until about 10 weeks after the beginning of the experiments the mice in both groups showed no abnormal clinical signs. After about 18 weeks, mice treated with 0.1μg/kg showed marked hypertrophy of the hearts; no pathological changes were seen in the hearts of the other mice. There was swelling or rupture of the endothelium of the capillaries and widening caused by exudation or collagen fibers in the interstitial space. Occasionally, degenerated or swollen mitochondria were prominent in the myocardium. Accumulations of platelets in the capillaries were frequently observed. Mice treated with low CTX dose showed no pathological changes even at the ultrastructural level until 40 weeks. tHUS CTX has a potent cumulative effect on the cardiac tissue.

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Ciguatera is one of the most serious tropical food poisonings of fish in the vicinity of coral reefs; its symptoms are neurological, gastrointestinal and cardiac (Baden,1983). Several toxins were isolated from contaminated fishes or cultured dinoflagellates and the chemical structures determined (Murata et al.,1989). Among them, CTX is the most potent.A feature of ciguatera is its obstinacy and recurrence of attacks (Bagnis,1968, Bagnis et al., 1979). Our study of short-term, successive administration of CTX at a low dose, confirmed that CTX has a cumulative effect on the cardiac tissue. In experiments reported here we re-examined the potency of the cumulative effects of the toxin on the mouse heart tissue.

MATERIALS AND METHODS

TOXIN

CTX used herein was isolated from reef snappers (*Lutjanus bohlar*) from Micronesia and Okinawa. Phycotoxin was purified as described by Legrand et al. (1989). One mouse unit is the minimal lethal dose of CTX 24hrs after i.p. administration into 20g mouse and is equivalent to 0.35μg/kg body weight (Yasumoto pers. comm.). Oral LD_{50} was not determined because the available dose of phycotoxin was limited.

EXPERIMENTAL ANIMALS

Male ICR mice (4 weeks of age weighing 20-23g) were obtained from Charles River Japan Inc., Tokyo. 40 mice were divided into 3 groups. Group 1: Five mice were given physiological saline with a stomach tube once a week for 40 weeks and served as control. Group 2: Twenty mice were given CTX (0.1μg/kg of body weight). Group 3: Fifteen mice were given low CTX (0.05μg/kg). Mice in groups 2 and 3 were given the phycotoxin in a similar manner to group 1. Two mice each from group 2 were sacrificed by cervical dislocation 5 hours after the intubation at the 12th, 14th, 18th and 23rd week from the beginning. After the treatment all surviving mice were fed a standard diet (CE-2, Nihon Clea Inc., Tokyo). Two mice from the group 3 were also killed and controlled in a similar manner to mice in group 2 at the 18th and 40th week.

MORPHOLOGICAL EXAMINATION

After necropsy all internal organs were fixed in 10% neutral formalin and embedded in paraffin. The slides for light microscopy were stained HE and PAS. For TEM, pieces of the heart, kidney, and liver were fixed by cold paraformaldehyde-glutaraldehyde solution (final concentration: 2%) and immersed in buffered 1% OsO_4 at room temperature. Then dehydrated in a series of graded ethanol, embedded in Epon 812, and cut with a diamond knife on a Porter II ultratome. The ultrathin sections were stained with uranyl

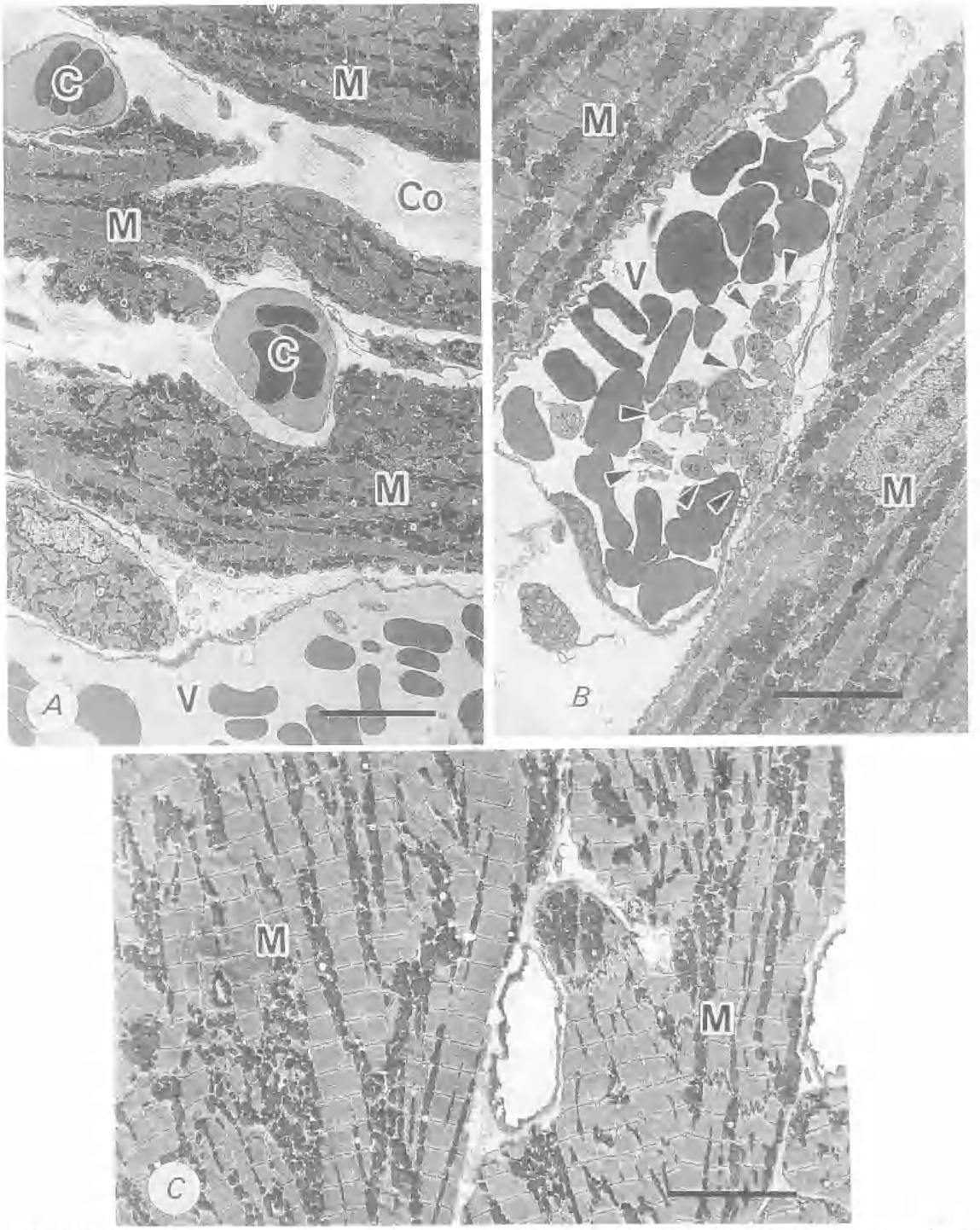

FIG. 1. A, Electron micrograph of heart from mouse given 0.1μg/kg CTX orally by intubations once a week for 25 weeks. Between cardiac muscles (M) are thick bundles of collagen (Co). Blood capillaries (C) are embedded in the collagen fibers. V: vein. Bar: 10μm. B, Electron micrograph of the heart from mouse given 0.1μg/kg CTX orally by intubations once a week for 25 weeks. In a small vein (V) a thrombus (arrow heads) is developing. M: cardiac muscle. Bar: 10μm. C, Electron micrograph of the heart from a mouse given 0.05 μg/kg CTX orally by intubation for 40 weeks. No discernible changes are seen. M: cardiac muscles. Bar: 10μm.

acetate and lead citrate and examined with a Hitachi H700H TEM.

RESULTS

Oral administration of CTX at a dose of 0.1μg/kg resulted in no abnormality, whereas i.p. injection of the same dose caused severe watery diarrhoea within 10 minutes. In contrast, mice injected i.p. with the phycotoxin at a dose of 0.05μg/kg induced no diarrhoea at all.

Even at the ultrastructural level a single oral dose of 0.1μg/kg of CTX produced no abnormal changes in the heart muscle. Long-term, intermittent administration of CTX at a dose of 0.05μg/kg induced no pathological changes in the heart tissue until 40 weeks. In contrast, intermittent administration of 0.1μg/kg CTX once a week for over 12 weeks resulted in severe morphological changes in heart tissue.

Mice given 0.05μg/kg CTX showed no abnormal behaviour through the experiment. Mice treated with CTX at an intermittent oral dose of 0.1μg/kg also showed no abnormality during the first 12 weeks. After that, however, shock often occurred shortly after administration of CTX. Usually the animals recovered spontaneously within 10 minutes. After 18 doses or at the 18th week two mice were sacrificed. Both ventricles of the animals were dilated at necropsy. Histopathologically, multiple single cell necroses were often seen in the mural or papillary muscles of the left ventricle. TEM examination showed swelling of myocardiac cells and edema between bundles of muscle fibres. Mitochondria in these cells became rounded and the matrix was electron-dense. Occasional dissociation of intercalated discs was noted. Blood capillaries were embedded by bundles of collagen fibers and electron-dense flocculent materials (Fig.1A). Capillaries and small veins were often occluded by accumulation of blood platelets (Fig.1B).

Mice given 0.05μg/kg CTX produced no changes in heart tissue until 40 weeks (Fig.1C).

DISCUSSION

The most prominent morphological changes after administration of CTX occurred in the heart muscles (Terao et al.,1990,1991,1992). Almost all cardiac muscle cells and the endothelium of blood capillaries in the cardiac interstitium were markedly swollen and ruptured with severity of change dependent on dose. These edema may be caused by the increased influx of Na^+ channels of cardiac muscle cells (Ohizumi,1990). In our previous report, short-term successive administration of CTX at low dose resulted in a cumulative effect of CTX on the mouse heart (Terao et al., 1992). In the present study, an intermittent dose resulted in similar severe morphological changes in the heart tissue. CTX may bind very tightly to Na^+ channels on the cardiac muscle cells or to those on the endothelium of the capillaries in the interstitium.

LITERATURE CITED

BADEN, D.G. 1983. Marine food-borne dinoflagellate toxins. Pp. 99–150. In G.H. Bourne & J.F. Danielli (eds) 'International Review of Cytology 82' (Academic Press: New York).

BAGNIS, R. 1968. Clinical aspects of ciguatera (fish poisoning) in French Polynesia. Hawaii Medical Journal 28: 25–28.

BAGNIS, R., KUBERSKI, T. & LAUGIER, S. 1979. Clinical observations on 3,009 cases of ciguatera (fish poisoning) in the South Pacific. American Journal of Tropical Medicine and Hygine 28: 1067–1073.

LEGRAND, A.M., LITAUDON, M., GENTHON, J.N., BAGNIS, R. & YASUMOTO, T. 1989. Isolation and some properties of CTX. Journal of Applied Physiology 1: 183–188.

MURATA, M., LEGRAND, A.M., ISHIBASHI, Y. & YASUMOTO, T. 1989. Structures of CTX and its congener. Journal of American Chemical Society 111: 8929–8931.

OHIZUMI, Y., SHIBATA, S. & TACHIBANA, K. 1981. Mode of a excitatory and inhibitory actions of CTX in the guinea pig vas deferens. Journal of Pharmacology and Experimental Therapeutics 221: 748–752.

TERAO, K., ITO, E. & YASUMOTO, T. 1990. Pathomorphological studies on experimental maitotoxicosis and ciguatoxicosis in mice. Pp. 55–70. In T.J. Tosteson (ed.), 'Ciguatera Puerto Rico 1990' (Polyscience Publication: Quebec).

TERAO, K., ITO, E. OARADA, M., ISHIBASHI, Y., LEGRAND, A.M. & YASUMOTO, T. 1991. Light and electron microscopic studies of pathologic changes induced in mice by CTX poisoning. Toxicon 29: 633–643.

TERAO, K., ITO, E. & YASUMOTO, T. 1992. Light and electron microscopic studies of the marine heart after repeated administrations of CTX or CTX-4c. Natural Toxins 1: 19–26.